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Prevalence of Antibodies to the Hepatitis E Virus in Pigs From Countries Where Hepatitis E Is Common or Is Rare in the Human Population

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Hepatitis E virus (HEV) is a very important public health concern in many developing countries where epidemics of hepatitis E are common. Sporadic cases of clinical hepatitis E not only occur in these countries but also occur uncommonly in patients with no known epidemiological exposure to HEV in industrialized countries. The source of infection in industrialized countries is unknown but it has been suggested that animals might serve as a reservoir for HEV in both settings. We recently identified and characterized an HEV strain (swine HEV) that infects large numbers of pigs in the United States. To assess the potential of pigs to serve as a global reservoir of HEV, we measured the prevalence of HEV antibodies in pigs in two countries where hepatitis E is endemic and two countries where it is not. Swine herds in all four countries contained many pigs that were seropositive for IgG anti-HEV, although the percentage of seropositive pigs varied greatly from herd to herd. A very limited number of pig handlers in the two endemic countries were also tested and most of them were found to be seropositive for HEV. The results from this study suggest that hepatitis E is enzootic in pigs regardless of whether HEV is endemic in the respective human population. ***J. Med. Virol. 59:297–302, 1999.*** Published 1999 Wiley-Liss, Inc.†

KEY WORDS: swine HEV; IgG anti-HEV; zoonotic; enzootic

INTRODUCTION

Hepatitis E virus (HEV), the causative agent of hepatitis E, was recently removed from the family *Caliciviridae* and is currently unclassified [Koonin et al., 1992; Pringle, 1998]. Hepatitis E is an important public health problem in developing countries [Arankalle et al., 1995; Purcell, 1996; Reyes, 1997]. The disease usually affects young adults and has a relatively high mortality in infected pregnant women [Hamid et al., 1996; Purcell, 1996; Hussaini et al., 1997; Reyes, 1997]. HEV is transmitted by the fecal-oral route, often causing water-borne epidemics. Hepatitis E is rarely diagnosed in industrialized countries, although anti-HEV antibodies were found in a significant proportion of individuals from some regions [Dawson et al., 1992; Herrera et al., 1993; Jardi et al., 1993; Paul et al., 1994; Johansson et al., 1995; Mast et al., 1997; Thomas et al., 1997]. It is not clear how such individuals became seropositive. Balayan et al. [1990] and Usmanov et al. [1994], respectively, reported that domestic swine and sheep were susceptible to infection with a strain of HEV endemic to central Asia. In addition, Clayson et al. [1995] reported that pigs were naturally infected with HEV in the Kathmandu Valley of Nepal where the disease is endemic. These data suggested that an animal reservoir for HEV may exist [Balayan et al., 1990;

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TABLE I. Prevalence of Antibodies to Hepatitis E Virus in Pigs and Pig Handlers From China

Sample source	Herd	Age	Number of samples tested	Number of samples positive for IgG anti-HEV (%)
Swine	A	1 month	11	6 (55)
	A	2 months	10	3 (30)
	B	3 months	10	0 (0)
	B	4 months	10	4 (40)
	B	5 months	10	4 (40)
	C	6 months	10	1 (10)
	C	Adult	11	4 (36)
	D[a]	Adult	10	0 (0)
Swine handlers			11	11 (100)
Blood donors			31	17 (55)

[a]Specific-pathogen-free swine.

Clayson et al., 1995; Reyes, 1997]. In fact, HEV antibodies have been detected in cows, primates, and rodents [Karetnyi et al., 1993; Tsarev et al., 1995, 1998; Maneerat et al., 1996; Favorov et al., 1998; Kabrane-Lazizi et al., 1999], suggesting that these animals have been infected by HEV or a related agent and that hepatitis E may be a zoonotic disease.

Recently, we identified and characterized a strain of HEV (swine HEV) that was recovered directly from pigs in the United States [Meng et al., 1997]. We also experimentally reproduced swine HEV infection in specific-pathogen-free (SPF) pigs and reisolated the virus from them [Meng et al., 1998a]. The swine virus appears to be highly infectious since the majority of pigs in the United States become infected by swine HEV at about 2 to 3 months of age. Although the infection is subclinical, microscopic evidence of hepatitis was found in naturally infected pigs and in some experimentally infected pigs. Swine HEV is genetically related to, but distinct from, most human strains of HEV [Meng et al., 1998b]. We recently showed that swine HEV can cross species barriers and infect rhesus macaques and chimpanzees, the most relevant surrogates for human infection [Meng et al., 1998b]. Experimental infection of nonhuman primates with swine HEV strongly suggested that swine HEV could infect humans. These data also suggest that an animal reservoir for HEV may exist even in a country where the disease is virtually unknown.

Recently, acute clinical hepatitis E was reported in two U.S. residents, one in Minnesota and one in Tennessee [Kwo et al., 1997; Schlauder et al., 1998; Erker et al., 1999]. The patient in Tennessee (US-2) had traveled to Mexico prior to the diagnosis of hepatitis but the patient from Minnesota (US-1) had no history of travel to an endemic region. The two U.S. strains of HEV (US-1 and US-2) were closely related to the swine HEV recovered from pigs in Illinois [Meng et al., 1998b; Schlauder et al., 1998]. The two U.S. human HEV strains had about 99% amino acid sequence identity with the swine HEV in ORF1, but only about 80% identity with other human strains of HEV from other countries. More importantly, we were able to infect SPF pigs experimentally with the US-2 human strain of HEV [Meng et al., 1998b], whereas we had been unable to infect them with two well-characterized human strains from Asia and Mexico, respectively [Meng et al., 1998a]. These data provide compelling evidence that a strain of HEV that is very similar to swine HEV can infect and cause hepatitis in humans.

The objective of the present study was to assess the prevalence of antibodies to HEV in pigs from other countries, either endemic or nonendemic for HEV in humans, in order to determine if infection of pigs with HEV is widespread and can occur in the absence of human HEV disease. We report here evidence for widespread HEV infection in pigs whether or not they are raised in a country endemic for HEV disease in humans.

MATERIALS AND METHODS

Serum Samples

Convenience serum samples were taken from pigs of different ages in four countries. Only a small percentage of pigs in each herd were tested. China and Thailand are both endemic for human HEV disease, while Korea and Canada are not. A limited number of serum samples from pig handlers in the two countries with endemic HEV disease were also collected.

Eighty-two serum samples were taken from pigs of various ages in four different commercial swine farms near Beijing, China (Table I). In addition, serum samples from 11 pig handlers and 31 normal blood donors in the same geographic area were collected (Table I).

Seventy-five serum samples were taken from pigs of various ages in four commercial swine farms located in different geographic regions in Thailand (Table II). In addition, sera from seven pig handlers were also collected.

Serum samples were collected from 140 pigs of various ages from several commercial swine farms in Korea (Table III).

Serum samples were collected from 400 pigs in 53 commercial swine farms in Quebec, Canada. These sera were taken from nursery pigs, adult boars, and sows (Table IV). An additional 312 serum samples were collected from nursery pigs, and a mixture of gilts and sows in Ontario (Table IV).

TABLE II. Prevalence of Antibodies to Hepatitis E Virus in Pigs and Pig Handlers From Different Regions of Thailand

Sample source	Herd[a]	Age	Number of samples tested	Number of samples positive for IgG anti-HEV (%)
Pigs	A[b]	1 month	10	0 (0)
	A	2 months	10	0 (0)
	A	3 months	10	9 (90)
	A	4 months	10	4 (40)
	B[c]	6 months	10	7 (70)
	B	Sows	10	2 (20)
	C[d]	Adult	5	1 (20)
	D[e]	Sows	10	0 (0)
Pig handlers		Adult	7	5 (71)

[a]All herds consisted of 1,000 or more sows and produced market pigs.
[b]Herd A is in the northeastern region of Thailand, is pseudorabies-free, and has its own seed-stock pigs imported from Denmark.
[c]Herd B is in the central region of Thailand, is pseudorabies-free, and its replacement pigs were from various sources in Thailand, including herd A.
[d]Herd C is in the northeastern region of Thailand (approximately 200 kilometers away from farm A), routinely imports replacement pigs from the largest pig company in Thailand, and had some seed-stock pigs imported from Ireland 2 years ago.
[e]Herd D is in the western region of Thailand.

TABLE III. Prevalence of Antibodies to Hepatitis E Virus in Pigs From Korea

Age	Number of samples tested[a]	Number of samples positive for IgG anti-HEV (%)
1 month	20	1 (5)
2 months	20	5 (25)
3 months	20	10 (50)
4 months	20	11 (55)
5 months	20	6 (30)
6 months	20	12 (60)
Sows	20	12 (60)

[a]Samples were categorized by age of pig and each age group represents multiple herds.

TABLE IV. Prevalence of Antibodies to Hepatitis E Virus in Pigs From Canada

Location of herd	Type of herd	Number of herds	Number of pigs tested	Number of pigs positive for IgG anti-HEV (%)
Quebec	Adults	16[a]	90	34 (38)
	Nursery	37[b]	310	82 (26)
Ontario	Adults	4[c]	82	12 (15)
	Nursery	10[d]	230	1 (< 1)

[a]The adult pigs consisted of 13 boar herds and 3 sow herds. Five herds of boars were seronegative and the percentage of seropositive boars in eight other herds ranged from 22% to 100%. Two herds of sows were seronegative and 50% of the sows in a third herd were seropositive.
[b]Thirteen herds were seronegative and the percentage of seropositive pigs in the remaining 24 herds ranged from 10% to 100%. Sera were collected from pigs 4 to 12 weeks of age.
[c]Adult pigs were a mixture of gilts (8 to 12 months old) and sows (1 to 3 years old).
[d]Nursery pigs were about 8 weeks of age.

ELISA for Detecting HEV Antibodies

The ELISA protocol standardized to detect anti-HEV in pigs is essentially the same as previously described [Meng et al., 1997, 1998a, 1998b]. These previous studies demonstrated the specificity and sensitivity of the ELISA based on the results obtained with known pre- and postinfection sera. The criteria for the ELISA positivity were described previously and resulted in a cutoff value of approximately 2.5 standard deviations above the mean absorbance value of the normal sera (Meng et al., 1997). Since the capsid antigen of Sar-55 is broadly reactive (Tsarev et al., 1993; Meng et al., 1997, 1998a, 1998b; Thomas et al., 1997), shares 92% of amino acid identity with swine HEV, and was the ELISA antigen originally used to identify swine HEV (Meng et al., 1997), it was used as the ELISA antigen in the serosurvey. HRP-conjugated goat antiswine IgG (H+L) (KPL, Gaithersburg, MD) was used as the secondary antibody. Preimmune and hyperimmune anti-HEV swine sera [Meng et al., 1997] were included as negative and positive controls, respectively. The protocol used for detecting HEV antibodies in human sera was essentially the same as the standardized ELISA for detection of HEV antibodies in nonhuman primates described previously [Tsarev et al., 1993, 1995]. Convalescent sera from a chimpanzee experimentally infected with HEV and preinoculation chimpanzee sera were included as positive and negative controls, respectively. All swine and human serum samples were tested in duplicate at a dilution of 1:100. About 66% of the positive pigs from these countries had an OD greater than 0.5, whereas 80% of the negative pigs had an OD less than 0.2 when the cutoff value was 0.33.

It is illegal to import swine sera containing infectious agents into the United States. Therefore, pig sera from China, Korea, and Thailand were sent directly to the U.S. Department of Agriculture Plum Island Foreign Animal Quarantine Facility for sterilization by irradiation before testing in the laboratory. The sera from Canada were not irradiated. Nine serially diluted positive swine sera with known anti-HEV ELISA titers were irradiated as a control for radiation damage. For eight of the nine samples, the OD value of the irradiated sample was within 15% of that of the nonirradiated sample. Three of the samples showed a decrease (5% to 10%) and five showed an increase (1% to 14%).

All of these samples had a preirradiation OD greater than 0.5 (0.548 to 1.709). The ninth sample went from 0.212 prior to irradiation to 0.321 after irradiation. These differences represent variations in the test. Therefore, irradiation had no detectable effect.

RESULTS

Prevalence of HEV Antibodies in Pigs and Pig Handlers From Two Countries That Are Endemic for HEV

Pigs of various ages from several commercial swine farms in China and Thailand were tested for the prevalence of IgG anti-HEV. In China, about 40% of the pigs older than 4 months of age were positive for anti-HEV (Table I). All 10 pigs 3 months of age were seronegative as were all 10 adult pigs from an SPF herd. All eleven pig handlers tested were seropositive for anti-HEV (Table I). However, 55% of 31 normal blood donors from the same geographic region were also positive for IgG anti-HEV.

Similar results were observed in pigs in different regions of Thailand (Table II). Pigs 2 months of age or younger were negative for IgG anti-HEV. However, about 20% to 90% of the pigs older than 3 months of age were seropositive. This is consistent with our previous finding that the majority of pigs in the United States were infected at 2 to 3 months of age. The percentage of seropositive pigs varied from herd to herd. To some extent this may reflect the small sample size. A very limited number of pig handlers also were tested and 71% (5/7) of them were positive for IgG anti-HEV. We were unable to obtain sera from normal blood donors in this region.

Prevalence of HEV Antibodies in Pigs From Two Countries in Which HEV Is Not Endemic

We showed previously that the majority of pigs from commercial swine farms in the midwestern United States were infected by HEV [Meng et al., 1997]. To assess the prevalence of HEV antibodies in pigs from other HEV nonendemic countries, we tested 852 pigs of various ages from more than 60 different farms in Canada and Korea.

One hundred and twenty pigs ranging from 1 to 6 months old from approximately 20 commercial swine farms and 20 adult sows from 3 swine farms in Korea were tested for anti-HEV (Table III). The serum samples were categorized by age of pig rather than by farm and so the percentage of positive pigs is an average. About 5% of pigs 1 month old were seropositive, whereas about 60% of the 6-month-old pigs and adult sows were seropositive.

We also tested 400 serum samples taken from nursery pigs, boars, and sows in 53 different swine herds in Quebec, Canada. As shown in Table IV, seropositive pigs were found in herds both of nursery pigs and of boars and sows. However, the percentage of seropositive pigs varied greatly from herd to herd, ranging from 0% to 100%. In addition, we tested 312 pigs from 10 swine herds in Ontario (Table IV). All the nursery pigs (about 8 weeks of age) except one were seronegative for anti-HEV. However, three herds of adult pigs (a mixture of gilts and sows) were seropositive for anti-HEV, although the nursery pigs in these herds were seronegative. Similar results were obtained with in pigs from Alberta and Saskatchewan (data not shown).

DISCUSSION

Clinical cases of hepatitis E are rarely reported in industrialized countries, although HEV antibodies are present in a significant proportion of individuals in these countries [Dawson et al., 1992; Herrera et al., 1993; Jardi et al., 1993; Paul et al., 1994; Johansson et al., 1995; Mast et al., 1997; Thomas et al., 1997]. Most clinical cases of hepatitis E reported in industrialized countries have been in travelers returning from endemic regions, although in some cases this risk factor was absent [Herrera et al., 1993; Jardi et al., 1993; Johansson et al., 1995; Kwo et al., 1997; Hsieh et al., 1998; Schlauder et al., 1998, 1999; Erker et al., 1999; Zanetti et al., 1999]. The recent identification of a case of acute hepatitis E in a U.S. patient with no history of travel to an endemic region [Kwo et al., 1997; Schlauder et al., 1998] suggests that the absence of travel to an endemic region does not necessarily exclude a diagnosis of acute hepatitis E. More recently, novel strains of HEV were discovered in other industrialized countries, including Italy, Greece, and Taiwan, in patients with no history of travel to endemic regions [Hsieh et al., 1998; Erker et al., 1999; Schlauder et al., 1999; Zanetti et al., 1999]. These new strains of human HEV had only about 80% nucleotide sequence identity with the known strains of HEV and with each other. The source of new HEV strains identified in industrialized countries is not clear, but given our discovery of swine HEV and its ability to infect across species, a zoonotic infection seems plausible.

HEV is ubiquitous in pigs in the midwestern United States [Meng et al., 1997] and also has been found in pigs from the Kathmandu Valley of Nepal [Clayson et al., 1995]. To ascertain whether HEV infection of pigs is widespread, we tested pigs from two hepatitis E endemic countries and two countries in which hepatitis E was not endemic. Our data suggested that HEV infection of pigs may be common worldwide. Swine herds in all four countries had pigs that were seropositive for anti-HEV. About 20% to 90% of the pigs older than 3 months of age were seropositive. This is consistent with our finding that the majority of pigs in the United States were infected at 2 to 3 months of age [Meng et al., 1997]. The relatively high prevalence of anti-HEV in pigs in the nonendemic countries suggests that virus infection may be enzoonotic even where it is not endemic for hepatitis E.

The high prevalence of swine HEV in pigs and the ability of swine HEV to infect across species may place swine practitioners, swine producers, and other pig handlers at possible risk of zoonotic infection by this virus. As a first step to assess the risks of HEV zoonosis, we tested a very limited number of pig handlers for

the prevalence of HEV antibodies in two hepatitis E endemic countries. All 11 pig handlers (100%) tested in one country and five of seven pig handlers (71%) tested from another country were seropositive for IgG anti-HEV. However, about 55% (17/31) of the normal blood donors in one of the endemic countries were also seropositive for IgG anti-HEV. Although the difference is statistically significant ($P < 0.05$, χ^2 test) and therefore suggestive, a definitive conclusion cannot be drawn because of the limited number of pig handlers tested. Nevertheless, our data indicated that the seroprevalence of IgG anti-HEV in pigs as well as in pig handlers was very high in these two HEV endemic countries. A much larger number of subjects, preferably in industrialized countries where the background of infection is low, is needed to determine the risk of transmitting HEV from pigs to humans.

Subclinical infection of humans with swine HEV could explain the relatively high prevalence of anti-HEV antibodies in some individuals in the United States and other industrialized countries. However, city dwellers in parts of the U.S. had a high prevalence of HEV antibodies [Mast et al., 1997; Thomas et al., 1997], and these individuals probably had no significant exposure to pigs, except possibly as food. Therefore, other species may serve as reservoirs for HEV. In addition to pigs, HEV antibodies have been detected in rodents, lambs, primates, cows, etc., but the agent responsible for seropositivity has not yet been identified. Recently, we found that the majority of wild-caught rats in three different geographic regions of the United States, including cities and rural areas, were positive for anti-HEV [Kabrane-Lazizi et al., 1999]. Although the natural history of HEV is not known, one might speculate that hepatitis E is a zoonosis and that HEV is constantly circulating among different animal species, including pigs, rats, and humans. The question then becomes whether there is a different strain for each animal species or whether a single strain circulates among all susceptible species within a geographic area. We were unable to infect pigs experimentally with known infectious stocks of Sar-55 or Mex-14 human strains of HEV [Meng et al., 1998a], suggesting that these epidemic strains may have a more limited host range than does swine HEV. It will be interesting to determine whether there are basic differences in host range or pathogenicity among strains circulating in countries where hepatitis E is endemic as compared to those where it is enzoonotic. Future studies in different regions of the world are needed to determine the natural history of HEV and to determine the genetic relationship between the virus infecting humans and the virus infecting other animal species within the same geographic area.

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